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## From oncogene to drug: development of small molecule tyrosine kinase inhibitors as anti-tumor and anti-angiogenic agents

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The confluence of two distinct but related activities in the past 10 years has dramatically accelerated efforts towards the discovery and development of novel drugs to treat cancer. The first is a rapidly emerging understanding that a number of distinct tyrosine kinases play roles in diverse but fundamentally important aspects of tumor progression (growth, survival, metastasis and angiogenesis). The second is the discovery that small molecule compounds have the capacity to potently and selectively inhibit the biochemical function of tyrosine kinases by competing for ATP binding at the enzyme catalytic site. These observations have been conjoined in major efforts to bring forward into clinical development novel cancer drugs with the potential to provide both clinical efficacy and improved tolerability. The focus of this review is on the development of small molecule tyrosine kinase inhibitors, and does not extend to other approaches that could be applied to disrupt the same pathways in clinical tumors (receptor and/or ligand-competitive antibodies, intrabodies, antisense ribonucleotides, ribozymes, phosphatase inhibitors or SH2/SH3-directed agents). Selected tyrosine kinase inhibitors, known or believed to be in development in cancer treatment trials, are summarized as are some of the key issues that must be addressed if these compounds are to be developed into clinically useful cancer chemotherapeutic agents. *Oncogene* (2000) 19, 6574–6583.

**Keywords:** tyrosine kinase inhibitors; anti-tumor; anti-angiogenesis

### Origin of species—brief overview of substrate-based inhibitors of protein tyrosine kinases

Among all non-traditional (non-DNA-directed) cancer targets for which pharmacological intervention is feasible, there are none that have generated as much widespread interest, and have invoked as much resource investment in both the public and private sectors in the past 7 years, as have the tyrosine kinases. Several excellent recent reviews have described the functions of various tyrosine kinases in the key pathways that drive tumor progression, from first genetic insult to disseminated disease (Hanahan and Weinberg, 2000; Hunter, 2000; Gibbs, 2000). Key among these are the receptor tyrosine kinases which initiate signal transduction in tumor cells or endothelial cells following the binding of the growth factors EGF, PDGF and VEGF. There are also several excellent reviews that provide detailed overviews of the work

accomplished to date to understand the molecular pharmacology of small molecule inhibitors of receptor tyrosine kinases (Sedlacek, 2000; Fry, 2000; Bridges, 1999; Levitzki, 1999; Lawrence and Niu, 1998). Without summarizing each of these important reviews, they provide an appropriate context for understanding the obstacles and triumphs that have led, very recently, to the first reproducible, objective clinical responses in cancer patients treated with tyrosine kinase inhibitors.

The catalytic function of protein tyrosine kinases involves the simple transfer of the gamma phosphate of ATP to hydroxyl group of a tyrosine residue of proteins (or peptides) encompassing a diversity of primary sequences and tertiary structures (Songyang and Cantley, 1998). Each of the substrates in the phosphotransfer reaction, the tyrosine hydroxyl group and ATP, represent reasonable pharmacological starting points for the design of substrate analogs and competitive inhibitors of tyrosine kinases. A diverse set of pharmacophores, including natural products (laven-dustins and erbstatins) and synthetic tyrosine mimetics, have all been characterized on the basis of their ability to competitively inhibit tyrosine kinase function (Levitzki, 1999). These compounds tended to have poor potency (particularly in cells), to yield relatively flat structure-activity relationships, and to be somewhat non-specific in their kinase inhibition (Fry, 2000). Attacking this reaction from the other side, by identifying compounds that mimic ATP, was originally thought to be even less tractable. As reviewed by Lawrence and Niu (1998), the theoretical obstacles were immense. First, the primary sequence of the ATP-binding pocket of all kinases is highly conserved, and therefore selectivity, if not specificity, represents a significant technical challenge. Secondly, the intracellular concentration of ATP can exceed 5 mM, particularly in tumor cells, while the  $K_m$  for ATP in most kinase active sites is in the micromolar range, thus ensuring full-time saturation by ATP. ATP-competitive inhibitors would need to exhibit at least nanomolar inhibitory kinetic constants to effectively compete in this circumstance (Lawrence and Niu, 1998). Finally, there are multiple non-kinase ATP-dependent enzymes important to normal physiology, and so an indiscriminant ATP mimetic would likely have toxicities that were pharmacologically and medically unacceptable.

This theoretical logjam was broken in convincing fashion when the tyrosine kinase inhibitory activities of anilinoquinazolines were first described in 1994 by three separate groups (Fry *et al.*, 1994; Ward *et al.*, 1994; Osherov and Levitzki, 1994). For example, the work of Fry *et al.* (1994) at Warner Lambert revealed that 4-anilinoquinazolines were potent (nM) inhibitors

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of the EGFR tyrosine kinase with good cell activity and profound biochemical selectivity relative to other kinases within the tyrosine kinase family. Further elaboration of structure-activity relationships rich in new possibilities resulted in ATP-competitive inhibitors of the EGFR tyrosine kinase with  $K_i$  values in the single digit picomolar range. It is interesting to note that the Michaelis-Menten equation could not be used to derive the  $K_i$  values of these molecules. So avid was the binding of compound to the ATP site, the conventional approximation that total and free enzyme concentrations were equivalent did not apply under these conditions. These accomplishments, which may be among the most important in pharmacology for the last 10 years, were largely achieved by empirical screening and iterative medicinal chemistry. Even more new chemotypes may emerge as structure-based design becomes more commonly applied to the identification of both active site- and allosteric site-directed inhibitors for an ever-widening slate of tyrosine kinase targets. While these early lead molecules had biopharmaceutical properties which were by-and-large incompatible with oral bioavailability and good duration of exposure *in vivo*, the results spurred on a number of groups, which have since identified and developed tyrosine kinase inhibitors with significant potential to treat clinical cancer.

#### Selected development candidates—updates

##### *PDGFR inhibitors: STI571 and SU101*

**STI571 (CGP57148B)** Among all of the candidates currently in clinical development, perhaps none has provided as much 'proof of concept' for the clinical efficacy and tolerability of small molecule tyrosine kinase inhibitors as has STI 571. Originally disclosed by Novartis as a multitrophic tyrosine kinase inhibitor, STI 571 was described by Druker *et al.* (1996); and Druker and Lydon (2000) as having potent activity *vs* the translocation product *bcr-abl*, the transforming tyrosine kinase found in virtually all CML cells expressing the Philadelphia chromosome (Kurzrock *et al.*, 1988; Kelliher *et al.*, 1990). The inhibition of *v-abl*, *bcr-abl* and PDGFR autophosphorylation by the 2-phenylaminopyrimidine STI 571 (Figure 1) at nanomolar concentrations was found to translate to both *in vivo* anti-tumor activity, and to the inhibition of clonogenicity of blasts from CML patients (le Coutre *et al.*, 1999; Druker *et al.*, 1996). The results of a clinical trial in which STI 571 was administered to CML and ALL patients expressing *bcr-abl* in their leukemic blasts were most recently summarized in May 2000 (Talpaz *et al.*, 2000). STI 571 was used to treat 33 acute leukemia patients, which included 21 myeloid blast crisis CML patients and 12 *bcr-abl*-positive ALL or lymphoid blast crisis CML patients. Clinical responses, as defined by a decrease in the percentage of patients achieving reduction in bone marrow blasts to 15% of pre-treatment levels, were observed in 55% of myeloid blast crisis patients, with complete responses in 22% of these patients. The response rates in patients with *bcr-abl* positive ALL and lymphoid blast crisis of CML were higher (82% with 55% complete responses), but all of the patients with lymphoid leukemias

relapsed on drug between 45 and 81 days. Of 19 responding patients, 10 experienced Grade 3–4 neutropenia. This response rate, and the incidence of Grade 3–4 toxicity, compares very favorably to the standard of care cytotoxic chemotherapies for CML. As such, more definitive trials assessing the efficacy and safety of STI 571 are ongoing in CML.

It is interesting to speculate as to the biochemical basis for both the efficacy and the toleration profile of STI 571. Two other tyrosine kinases potentially inhibited by STI 571, *c-kit* and PDGFR, are both believed to play important roles in maintaining bone marrow stroma–progenitor cell interactions (Ashman, 1999; Sungaran *et al.*, 2000). Thus, inhibition of *c-kit* and PDGFR could also account for some of the compelling clinical activity of STI 571 in CML, as well as for its toxicity profile (neutropenia). Treatment of a *c-kit* expressing a human myeloid leukemia cell line, M-07e, with STI 571 before stimulation with *kit* ligand inhibited *c-kit* autophosphorylation, activation of mitogen-activated protein (MAP) kinase, and activation of Akt, with an  $IC_{50}$  of 100 nM (Heinrich *et al.*, 2000). STI 571 was even more potent in a human mast cell leukemia cell line (HMC-1) expressing an activated mutant form of *c-kit*. Similar results have also recently been reported in non-hematopoietic tumor cells (Wang *et al.*, 2000). The efficacy and safety hypotheses for inhibition of *c-abl* in CML may perhaps only be addressed with a more selective *abl* tyrosine kinase inhibitor. Given the apparent therapeutic benefit of STI 571, this may be largely an academic question, but one with important implications as one tries to rationalize the desired selectivity profiles of tyrosine kinase inhibitors most likely to generate both efficacy and safety in humans.

**SU101 (leflunomide; HWA 486)** Leflunomide was originally described and developed as an inhibitor of dihydroorotate dehydrogenase, a key enzyme in the *de novo* synthesis of pyrimidines, for use as an immunosuppressive or anti-arthritis agent (Bartlett and Schleyerbach, 1985; Kuo *et al.*, 1996). Leflunomide has shown significant activity as a treatment for rheumatoid arthritis (Smolen and Emery, 2000; Cohen *et al.*, 2000b), and was launched by Aventis as Arava® in the US and elsewhere beginning in 1998. Extending the work of others (Mattar *et al.*, 1993; Xu *et al.*, 1995), Shawver and co-workers reported that micromolar concentrations of leflunomide inhibited the autophosphorylation of the tyrosine kinase receptors for PDGF and VEGF (Shawver *et al.*, 1997). The compound was also effective at blocking mitogenesis stimulated by both PDGF and EGF, but exogenous uridine could not reverse the effect of leflunomide on PDGF mitogenesis, suggesting that inhibition of the receptor tyrosine kinase, and not inhibition of pyrimidine pools, was a key pharmacological activity. The inhibition of EGF-induced mitogenesis by leflunomide was reversed in part by uridine (Shawver *et al.*, 1997), despite the fact that leflunomide and close-in analogs also have inhibitory activity *vs* the EGFR tyrosine kinase (Ghosh *et al.*, 1999).

Leflunomide/SU101 is clearly a tyrosine kinase inhibitor with multiple biochemical effects, and readily generates a predominant active metabolite (SU0020 or A771726; Figure 1) that has a complex inhibitory profile of its own (Hamilton *et al.*, 1999). SU 101 was,

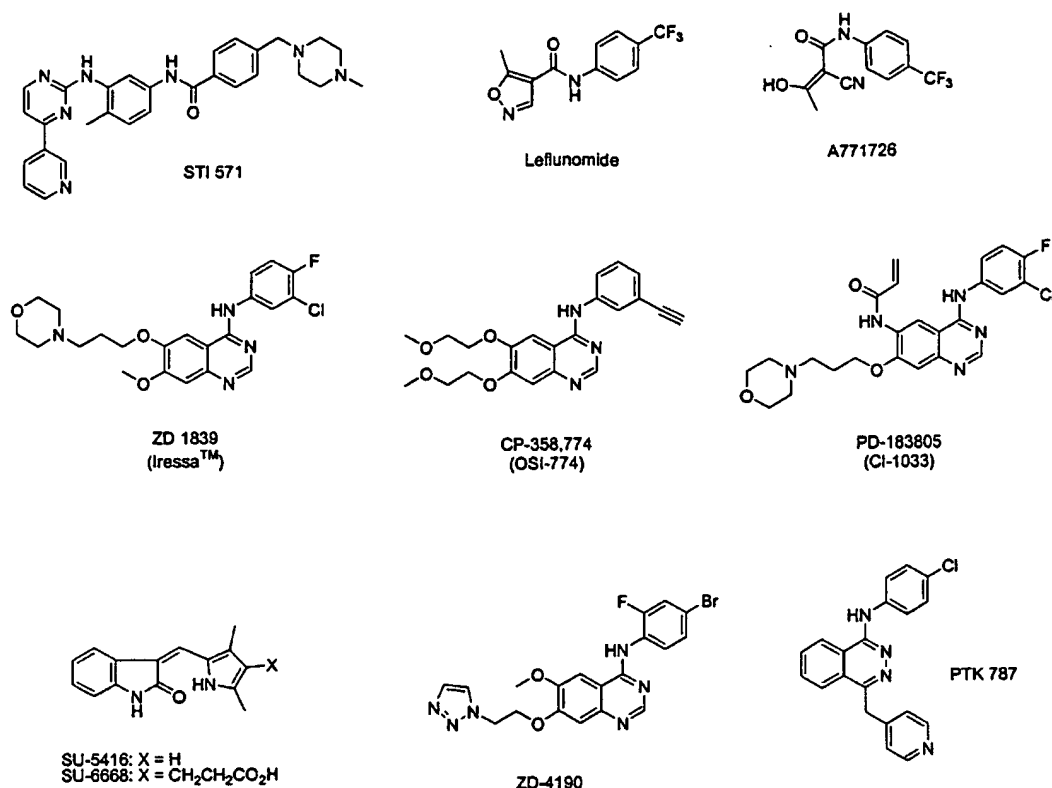


Figure 1 Structures of selected tyrosine kinase inhibitors in clinical development for cancer

nonetheless, progressed into clinical trials by SUGEN (now part of Pharmacia). A Phase I study in cancer patients revealed that SU101 was well-tolerated as a 24 h continuous i.v. infusion at doses up to 443 mg/m<sup>2</sup>/wk. At this dose, the plasma concentration of the active metabolite was maintained at levels sufficient to block both PDGFR and EGFR signaling, as well as pyrimidine biosynthesis (Eckhardt *et al.*, 1999). Toxicities were relatively minor (Grade 1–2 nausea, vomiting and fever in approximately 20% of all courses given). Surprisingly, hematopoietic toxicities and hemolysis, which had been noted in the preclinical experience with SU101, were not seen in this Phase I population. One partial response was seen in 26 patients receiving an average of two courses each; the responding patient received 13 courses (52 infusions) to treat an anaplastic astrocytoma, and had a notable (>50%) reduction in one measurable lesion (Eckhardt *et al.*, 1999). SU101 has been reported to be in advanced trials for multiple solid tumor types, but recent disclosures (Garber, 2000) indicate that Phase III trials in at least one tumor type (glioblastoma) have been abandoned. The status of other trials (ongoing Phase II trials for ovarian and NSCLC; planned Phase III trials for prostate, colon and NSCLC) is uncertain at the present time.

**EGFR inhibitors:** Iressa® (ZD1839), OSI-774 (CP-358,774) and CI-1033 (PD183805)

**Iressa® (ZD1839)** While STI 571 has provided notable clinical proof-of-concept for the clinical efficacy and safety of tyrosine kinase inhibitors, the early

clinical findings with AstraZeneca's ZD1839 (Iressa®) have been equally compelling. The pharmacological characteristics of Iressa® were first described in 1996 (Wakeling *et al.*, 1996; Woodburn *et al.*, 1997) as a potent and selective inhibitor of the EGFR tyrosine kinase. This quinazoline-based compound (Figure 1) is an ATP-competitive inhibitor of the EGFR tyrosine kinase (IC<sub>50</sub> 25 nM) with 50-fold selectivity relative to closely homologous *erbB* family members (IC<sub>50</sub> for *erbB2* 1–3 μM) and even greater selectivity for more divergent tyrosine kinases. It demonstrates good cellular potency (80 nM IC<sub>50</sub> for inhibition of EGF-dependent mitogenesis) and robust, dose-dependent anti-tumor efficacy in a variety of human tumor xenografts (Woodburn *et al.*, 1997). These results have been most recently extended to show that Iressa® has *in vivo* efficacy in a diverse human tumor xenograft models both with (Ciardello *et al.*, 2000) and without (Sirotnak *et al.*, 2000) highly activated EGFR signaling pathways. Of equal interest are the observations that Iressa® combines with standard cytotoxic agents (platinums, taxanes, topoisomerase I inhibitors, etc.) to produce additive or supra-additive anti-tumor efficacy *in vivo* without exacerbation of the toxicity of the co-administered cytotoxics. The findings that tumor EGFR density does not predict efficacy when the compound is used in conjunction with cytotoxic agents have significantly impacted the development strategy employed by AstraZeneca as Iressa® moves towards pivotal clinical trials.

Multiple Phase I trials with Iressa® have been summarized, and the results revealed reasonable pharmacokinetics, good toleration and the first signs

of clinical efficacy when used as a single agent in patients with advanced disease (Ferry *et al.*, 2000; Baselga *et al.*, 2000; Kelly *et al.*, 2000). Following oral administration of a single dose (50 mg), maximum plasma drug concentrations (mean 45 ng/ml) occurred 1–5 h post-dose. The mean terminal  $t_{1/2}$  was 34 h. Inter-subject variability in exposure was significant following single and multiple administration (up to sevenfold at each dose level), but exposure increased proportionally with dose, with no apparent change in terminal  $t_{1/2}$  across the dose range tested (Kelly *et al.*, 2000). In a larger dose-escalation trial, Ferry and collaborators administered Iressa® at doses of 50–700 mg once daily, given orally for 14 days followed by 14 days of observation (Ferry *et al.*, 2000). In total, 64 patients with advanced disease, who had each progressed while on prior chemotherapy, completed 145 cycles.  $C_{max}$  and  $AUC_{0-24h}$  were proportional across the entire dose range (mean values 113–2255 ng/ml and 1.8–38.5 mg.h/ml, respectively). As in single dose studies, Iressa® showed a long terminal elimination half-life (mean of 46 h). Iressa® was very well-tolerated in this study; the most common adverse events were diarrhea and acne-like skin rash (Grade 1–2). Acne-like skin rashes have emerged as a common, mechanism-based adverse event for EGFR inhibitors, but the specific toxicological effect in the skin is not yet well understood. Grade 3–4 adverse events were shown to be rare with Iressa® treatment, and were generally ascribed to disease progression. The dose-limiting toxicity, defined at the 700 mg dose level, was Grade 3 diarrhea (Ferry *et al.*, 2000).

A compelling level of efficacy was also revealed in these early trials (Ferry *et al.*, 2000). Anti-tumor responses were most evident among the 16 NSCLC patients treated with Iressa®—two had an objective partial response, two patients had significant regression of disease and two patients had stable disease. Similar pharmacokinetic and safety profiles were noted in a separate study (Baselga *et al.*, 2000), one that also revealed the potential for efficacy from Iressa® in patients with advanced prostatic and head-neck cancers. These early results added importantly to the proof-of-concept that selective tyrosine kinase inhibitors could have significant single agent efficacy, as measured by objective tumor regressions, in patients with advanced disease. The clinical observations have therefore recapitulated the pre-clinical data showing that Iressa® increased apoptosis and regressions in human tumor xenograft models (Ciardello *et al.*, 2000).

The Iressa® data indicate that the efficacy of these agents can be measured using more classically defined clinical endpoints. There will undoubtedly be significant value in the use of pharmacodynamic and surrogate endpoints to guide dose-intensification or to pre-select patients for whom other tyrosine kinase inhibitors might represent the most promising treatment option. Pharmacodynamic endpoints have not played a major role in the early development of EGFR tyrosine kinase inhibitors, despite the fact that several reasonable options exist, including both invasive techniques (direct measurement of tumor-derived or normal tissue-derived EGFR phosphotyrosine, phosphorylation of down-stream signaling molecules; apoptosis markers) and non-invasive techniques such as PET imaging of metabolically modulated tumors

(Pollack *et al.*, 1999; Goss *et al.*, 2000; Allen *et al.*, 2000). Given the overall safety and toleration profile of Iressa®, AstraZeneca has committed to an aggressive development strategy, which includes two large Phase III studies to assess the use of Iressa® in combination with cis- or carbo-platinum plus a taxane or gemcitabine in first-line therapy for NSCLC (trials 14 and 17), as well as a Phase II trial (trial 16) to confirm the single agent activity of Iressa® in patients with advanced NSCLC (Kelly *et al.*, 2000). It is important to note that these trials do not call for a prospective selection for patients with tumors with some pre-defined level of EGFR over-expression. All epithelial tumors express some EGFR, and in the disease target here, NSCLC, tumors often present with a high proportion of EGFR over-expression (up to 80–90% in advanced disease). The strategy is also consistent with pre-clinical data suggesting that efficacy in drug combinations may not be determined in large part by the level of EGFR over-expression in tumors (Sirotnak *et al.*, 2000). Results are expected from these pivotal trials in a late-2001 or early-2002 timeframe.

**OSI-774 (CP-358,774)** CP-358,774 is also a potent and selective quinazoline-based inhibitor of the EGFR function (Figure 1). This compound is a reversible, ATP-competitive inhibitor ( $IC_{50}$  of 2 nM) of the EGFR tyrosine kinase, with greater than 500-fold selectivity against other tyrosine kinases, such as the closely related *erbB2* kinase, as well as *v-src*, *c-abl* and the insulin and IGF-1 receptors, (Moyer *et al.*, 1997). CP-358,774 inhibits the autophosphorylation of the EGF receptor in a variety of EGFR over-expressing tumor cells ( $IC_{50}$  = 20 nM), and produces cell cycle arrest and apoptosis in multiple cell types (Moyer *et al.*, 1997; Barbacci *et al.*, 1997; Iwata *et al.*, 1997). *In vivo*, CP-358,774 effectively inhibits EGFR-specific tyrosine phosphorylation in human tumor xenografts ( $ED_{50}$  of 10 mg/kg p.o. when given as a single dose) with significant duration of action; daily dosing produces substantial growth inhibition and regressions in human tumor xenografts (Pollack *et al.*, 1999). Moreover, the dose-response for tumor growth inhibition shows good agreement with the dose-response for inhibition of EGFR-phosphotyrosine in tumors from treated animals. As with Iressa®, CP-358,774 was found to generate additive anti-tumor activity when used in combination with cis-platinum and other cytotoxic agents, without exacerbating the toxicities of the other chemotherapeutics (Pollack *et al.*, 1999).

Clinical studies with CP-358,774 have revealed that the agent is well-tolerated at oral doses that achieve plasma concentrations projected to be required for anti-tumor efficacy in humans (400–500 ng/ml). In one study, escalating doses were administered orally once every week (Karp *et al.*, 1999). Eighteen patients with advanced solid tumors were treated at five doses (100–1000 mg) for a maximum period of 24 weeks. Toxicities were observed only at doses higher than 200 mg/week, and included mild fatigue, Grade 2 maculopapular (acneiform) rash, Grade 2 nausea, and Grade 2 diarrhea. Like Iressa®, CP-358,774 exhibited intra- and inter-subject variability in exposure, but dose-proportional increases in exposure were observed throughout the 100–1000 mg weekly dose range. During the first 24 h following a single dose, the  $C_{avg}$

(0.9–4.8 mg/ml for 100–1000 mg doses, respectively) was some two- to 10-fold above the projected efficacious plasma concentration. No maximally tolerated dose or dose-limiting toxicity was discerned in this study. In a second Phase I study (Siu *et al.*, 1999), patients were given CP-358,774 tablets in a variety of dose schedules, culminating in daily dosing at the maximally tolerated dose. The target  $C_{avg}$  of 400–500 ng/ml was achievable at doses at and above 100 mg/day on a well-tolerated schedule ( $C_{avg}$  values following continuous daily dosing at the 50, 100 and 200 mg/day levels were 432, 973 and 2120 ng/ml, respectively). Dose-limiting diarrhea was encountered at the 200 mg/day level. An intermediate dose of 150 mg/day was subsequently defined as the maximally tolerated dose (two of three patients had Grade 1 diarrhea with loperamide support).

Siu and co-workers also made efforts to understand the 'characteristic' Grade 1–2 acneiform rash seen in patients treated with CP-358,774, which was limited to regions of the upper body where adolescent acne is usually manifest (face, back and scalp). Histopathology of skin biopsies showed subepidermal neutrophilic infiltration and epidermal hyperproliferation (Siu *et al.*, 1999). While the precise cytopathic basis for the acneiform rash has not yet been determined, the consistent clinical observations with three different agents targeting EGFR function (CP-358,774, Iressa® and Imclone's C-225 antibody) suggest that this is a mechanism-based finding (Siu *et al.*, 1999; Ferry *et al.*, 2000; Cohen *et al.*, 2000b). Skin changes are consistently noted in preclinical studies with rodents exposed to CP-358,774 for extended dosing periods, and these toxicological results are analogous to the skin changes seen in the waved-2 mouse, which has a mutated and marginally functional EGFR tyrosine kinase (Luetteke *et al.*, 1994).

Early efficacy readouts from ongoing Phase II clinical trials with CP-358,774 have been compelling. The agent appears to have a broad potential to treat a variety of human solid tumors, including NSCLC, breast, ovarian and squamous head and neck tumors (Bonomi *et al.*, 2000; Allen *et al.*, 2000; Siu *et al.*, 2000; Hammond *et al.*, 2000). For example, in 34 NSCLC patients who had failed prior chemotherapy, daily oral doses of 150 mg CP-358,774 were well-tolerated, with a maculopapular (acneiform) rash being the most common adverse event reported. In 56 total patients evaluable for tumor response, there have been six partial responses in the lung and/or liver at 8 weeks and several patients with stable disease (Bonomi *et al.*, 2000). In 71 patients with refractory squamous carcinomas of the head and neck, CP-358,774 was again found to cause a reversible acneiform rash and Grade 1–2 diarrhea. Of 78 patients evaluable for response, there have been at least eight confirmed partial responses and 23 patients with stable disease (Siu *et al.*, 2000). These preliminary results indicate that CP-358,774 is generally well-tolerated and demonstrates evidence of single agent anti-tumor activity in patients with recurrent head and neck cancer, as well as in treatment-refractory NSCLC.

Due to significant interests in both CP-358,774 and CI-1033, Pfizer was directed to divest one of these two agents as a condition of their acquisition of Warner Lambert in 2000. As such, Oncogene Science (OSIP)

has taken over complete responsibility for the development of CP-358,774, which is now formally referred to as OSI-774.

**CI-1033 (PD183805)** As described above, the selective and reversible inhibitors of the EGFR tyrosine kinase appear to offer the promise of therapeutic efficacy coupled to reasonable tolerability. It is important to note, however, that the therapeutic index of neither Iressa® nor CP-358,774 has yet to be fully elaborated, and that there may be significant proximity between the maximally tolerated doses and the efficacious doses for both agents. Moreover, the efficacy of neither agent has yet to be established in a blinded, placebo controlled study. As such, there continues to be an opportunity to discover and develop distinctly different EGFR tyrosine kinase inhibitors with even greater potential for efficacy and a broader spectrum of activity. CI-1033 is one such distinctly different development candidate. As recently reviewed by David Fry of the former Warner Lambert organization, signaling through the *erbB* family of tyrosine kinase receptors often involves complex cross-talk among the members of that receptor family (Fry, 2000). The four family members (EGFR or *erbB*; *erbB2*, *erbB3* and *erbB4*) are known to intensify their kinase-dependent transforming signals via the formation of heterodimers with each other (Tzavaras *et al.*, 1996). There is, therefore, a compelling rationale to consider the potential utility of nonspecific but selective inhibitors that effectively block the function of the *erbB* family but do not inhibit more structurally diverse tyrosine kinases.

There is also a strong rationale to consider irreversible tyrosine kinase inhibitors. The reversible inhibitors have apparently generated clinical efficacy with dosing regimens designed to maintain plasma concentrations at fairly high levels for extended periods of time. The optimal dosing paradigm for an irreversible inhibitor would be less likely to require prolonged exposure. Moreover, the 'absolute finality' (Fry, 2000) of the irreversible inhibitors could conceivably provide significant advantages in terms of antitumor efficacy. To be balanced, a multi-tropic and irreversible inhibitor would also have the potential to generate a toxicity profile that was different and, perhaps, without advantages relative to the more selective, reversible inhibitors. Preclinical data suggest that irreversible EGFR tyrosine kinase inhibitors can generate significant efficacy with good toleration (Vincent *et al.*, 1999), but the ultimate utility of these agents can only be determined in clinical trials.

Homology modeling of ATP binding to the pocket of EGFR suggested that the thiol of *cys773* would be a key potential site for attack by a rationally designed irreversible ATP-mimetic. One compound containing an acrylamide functionality at the six position of the 4-anilinoquinazoline nucleus (Figure 1) was found to have a profoundly rapid onset and long-lasting inhibition of both EGFR and *erbB2* in tumor cells, and to be selective relative to non-*erbB* tyrosine kinases (Fry *et al.*, 1998). When compared to very closely related reversible analogs (in which the acrylamide double bond was reduced), the 6-substituted irreversible analogs were more potent *in vitro* and had significantly greater efficacy *in vivo*. Further improve-

ments (addition of substitutions which also improved water-solubility) led to the elaboration of PD 183805/CI-1033 (Figure 1). Like its predecessors, this compound has excellent (low nM) potency against *erbB2* and EGFR in both enzyme- and cell-based assays (Sherwood *et al.*, 1999). Consistent with a predicted advantage relative to reversible inhibitors, CI-1033 potently inhibits human tumor xenografts when dosed as infrequently as once per week, and a single dose eliminated the level of EGFR phosphorylation in tumors for longer than 72 h (Vincent *et al.*, 1999). Like CP-358,774, CI-1033 combines well in drug combinations with cytotoxic agents. Given 24 h after gemcitabine, CI-1033 produced a significant increase in the apoptotic fraction in tumors over treatment with either drug alone (Nelson and Fry, 2000). CI-1033 also effectively decreased the clonogenicity of human tumor cells taken from patients (Medina *et al.*, 2000), with notable responses seen in breast (67%), NSCLC (60%) and ovarian cancer specimens. CI-1033 Phase I clinical trials have recently been initiated, but data on pharmacokinetics or safety have not yet been disclosed.

#### *Small molecule tyrosine kinase inhibitors targeting angiogenesis pathways*

There are multiple tyrosine kinase receptors which appear to have key roles in the generation of new tumor blood vessels and, as such, represent reasonable targets for cancer chemotherapy (for excellent recent reviews, see Cherrington *et al.*, 2000; Randal, 2000; Thompson *et al.*, 1999; Hamby and Showalter, 1999). Included among the key tyrosine kinase targets that have generated the most interest in the scientific and patent (Connell, 2000) literature are PDGFR, VEGFR, FGFR and tie-2. The key development candidates targeting PDGFR, STI 571 and SU101, were described above, though neither compound is likely to reveal the clinical utility of PDGFR-directed inhibition of angiogenesis due to their multiple mechanisms of action. Agents that selectively target FGFR and tie-2 are not known to be in development, though several drugs targeting VEGFR have inhibitory activity vs FGFR. As such, the focus of the remainder of this overview will be on the clinical candidates targeting VEGFR. Two high affinity receptors for VEGF have been identified and characterized on human endothelial cells, *flt-1* and KDR. KDR appears to be expressed primarily on activated endothelial cells and is thought to be more of a key driver of mitogenic responses commonly found in neovascularizing tumors, while *flt-1* is expressed on multiple other cell types (Plate *et al.*, 1994; Wedge *et al.*, 2000a). For the purposes of this review, the terms KDR and VEGFR will be used interchangeably, unless otherwise specified.

**SU 5416 and SU 6668** The former SUGEN organization (now part of Pharmacia) has clearly set the early pace in the race to identify and develop inhibitors of the VEGFR tyrosine kinase. Efforts towards this end have initially focused on the indolin-2-one pharmacophore (Figure 1). Among the earliest compounds of this class was SU 5416, which was found to be a potent inhibitor of the kinase activities of both VEGFR and PDGFR. Inhibition of these two tyrosine kinases was found to be competitive with ATP, but the inhibition

of FGFR, which occurred at SU 5416 concentrations some 100-fold higher, was found in kinetic experiments to be 'mixed' competitive and non-competitive (Mendel *et al.*, 2000). It has been speculated that the latter result is due to specific biopharmaceutical properties of the compound, which is both lipophilic and potentially reactive in nature. Consistent with this concept are preliminary observations that the inhibition of VEGF-dependent endothelial cell proliferation by SU 5416 has both a rapid onset and a pseudo-irreversible behavior which may be due to high intracellular levels of compound (Mendel *et al.*, 2000). Inhibition of endothelial cell proliferation translated to anti-tumor efficacy in a number of human xenograft and rodent tumor models (Fong *et al.*, 1999). In these studies, no data were generated to relate drug exposure (said to be very short-lived in rodents), or biochemical inhibition of VEGFR or PDGFR, to anti-tumor efficacy. Interestingly, the efficacy of SU 5416 was found to be greater in slower-growing vs faster growing solid tumor xenografts, which led Fong *et al.* (1999) to speculate that SU 5416 might bind preferentially to resting vs activated tyrosine kinases on endothelial cells. This would be at odds with other data suggesting that quinazolines bind more avidly to activated kinases (Levitzi and Bohmer, 1998) but, if true, may bode well for human efficacy in a majority of clinical settings.

Phase I studies were carried out in 69 advanced disease patients, with SU 5416 dosed i.v. twice weekly. Patients were treated at 13 dose levels between 4.4–190 mg/m<sup>2</sup>/day; at the highest dose, a dose limiting toxicity (projectile vomiting) was observed (Rosen *et al.*, 1999). Induction of metabolism was noted in all patients, either due to the parent drug, a metabolite or dexamethasone premedication, and the elimination half-life was found to be 55 min (Cropp *et al.*, 1999). Early signs of efficacy were also apparent, with objective responses seen in three patients (Kaposi's sarcoma, metastatic basal cell and colorectal cancer); seven patients remained on study for more than 6 months, while two remained on study for greater than 18 months (Rosen *et al.*, 1999; Mendel *et al.*, 2000). Given these results, SU 5416 has been advanced into multiple Phase II and III at an initial recommended dose of 145 mg/m<sup>2</sup>, which is sufficient to produce systemic exposure comparable to what was required to yield effective tumor growth inhibition in animals (Cropp *et al.*, 1999). This dose is also within 30% of the human maximally tolerated dose (190 mg/m<sup>2</sup>). The ongoing development plan includes large studies in NSCLC and colorectal cancer to assess the efficacy of SU 5416 both as a single agent and in combination with standard chemotherapies (Mendel *et al.*, 2000).

A related agent in development, SU 6668 (Figure 1), combines a less selective inhibitory profile (inhibition of FGFR in addition to PDGFR and VEGFR) with a more favorable biopharmaceutical profile (Laird *et al.*, 2000). SU 6668 has a significantly lower K<sub>i</sub> for PDGFR relative to VEGFR or FGFR (8 nM vs 2.1 and 1.2 μM, respectively), a result which appeared consistent with homology models of the respective active sites, but inconsistent with the cellular effects of SU 6668 (VEGFR-stimulated mitogenesis of endothelial cells much more potently inhibited relative to either PDGFR or FGFR) (Laird *et al.*, 2000). Like

SU5416, SU6668 was found to be potent and efficacious in a variety of tumor models. Unlike SU5416, which was dosed i.p. in a DMSO-based vehicle, efficacy was achievable with SU6668 when dosed orally each day in a cremaphore-based vehicle. In Phase I studies, SU6668 was administered orally once daily to 16 patients with advanced malignancies, at dose levels between 100–1600 mg/m<sup>2</sup>/day (Rosen *et al.*, 2000). Nine of 16 patients remained on study for up to 28 weeks while the remaining seven patients had progressive disease. Dose limiting toxicities were not observed, and dose escalation was said to be ongoing. Two patients at 1600 mg/m<sup>2</sup> developed liver function abnormalities, but both had potentially confounding liver disease. Other possible drug related toxicities included nausea, headache, fatigue and changes in bowel movements. Pharmacokinetic data suggested that SU6668 had a moderate-high clearance (78 l/day/m<sup>2</sup>) and a somewhat improved elimination half-life of 2.5 h relative to SU5416 (Rosen *et al.*, 2000). Phase II studies in multiple tumor types have apparently been initiated.

**ZD4190 and ZD6474** ZD4190 is a quinazoline-based VEGFR inhibitor (Figure 1) said to have entered Phase I in early 2000. ZD6474 is thought to be from the same structural class, but AstraZeneca has not yet disclosed the specific structure. ZD4190 inhibits both KDR and *flt-1* (IC<sub>50</sub> values of 29 and 708 nM, respectively), and much less potent at inhibiting FGFR (approximately 200-fold relative to KDR). The compound is also 30-fold more potent at inhibiting VEGF-mediated endothelial cell growth relative to FGF-stimulated cell growth (IC<sub>50</sub> values of 50 and 1530 nM, respectively) (Wedge *et al.*, 2000a). *In vivo*, the compound was found to inhibit capillary invasion of cartilage (increased epiphyseal growth plate area), and to inhibit the growth of four human tumor xenografts in a dose-dependent manner with daily oral administration (Wedge *et al.*, 2000a). Direct measurements of tumor vascular endothelial permeability, using contrast medium-enhanced MRI indicated that acute ZD4190 treatment produced measurable changes in vascular permeability at doses which yielded anti-tumor activity during chronic administration (Wedge *et al.*, 1999). ZD6474, the second putative development candidate, is unique among small molecule angiogenesis inhibitors, in that it is found to induce significant regressions in PC-3 tumors of varying size, with greatest effects being produced in the largest tumors (Wedge *et al.*, 2000b). An intermittent ZD6474 treatment schedule, involving withdrawal of compound for 4 weeks, revealed that tumor re-growth could be

halted and marked regressions could again be induced in these tumors upon re-treatment. While the pre-clinical data for both compounds appear to be very promising, Phase I results for neither ZD4190 nor ZD6474 have yet been disclosed.

**PTK 787** Novartis is reported to be developing PTK 787, which has an anilinothalazine pharmacophore (Bold *et al.*, 2000) related to but distinct from the quinazolines described above (Figure 1). The compound is a potent inhibitor of both major human VEGFR (IC<sub>50</sub> values of 37 and 77 nM for KDR and *flt-1*, respectively) and, like STI 571, it provides potent (sub-micromolar) inhibition of PDGFR and *c-kit* but does not inhibit *v-abl*, EGFR or FGFR (Wood *et al.*, 2000). PTK 787 inhibits VEGF-induced KDR auto-phosphorylation and mitogenesis, and promotes endothelial cell apoptosis, at a similar concentration (Wood *et al.*, 2000). The compound also has good biopharmaceutical properties (plasma concentrations >1 µM 8 h after administration of a 50 mg/kg oral dose to mice), and impressive antiangiogenic (ED<sub>50</sub> <12.5 mg/kg/day for inhibition of angiogenesis in a s.c. growth factor implant model) and anti-tumor activity (significant growth inhibition in six different human tumor xenograft models at daily oral doses of 25–75 mg/kg) (Wood *et al.*, 2000). A key issue in the field of anti-angiogenesis research has long been the fear that inhibition of tumor angiogenesis would also impair normal angiogenesis, such as that in wound healing. Given that most solid tumors are managed using multi-modality treatments that include surgery, this has been a theoretical limitation to inhibitors of angiogenesis. Interestingly, PTK 787 appears to have much less efficacy as an inhibitor of physiological angiogenesis of wound healing than as an effective blocker of tumor angiogenesis. Daily dosing of rats up to 50 mg/kg day did not impair the healing or decrease the tensile strength of full-thickness incisional wounds (Wood *et al.*, 2000). Data on the antitumor activity of PTK 787 were recently extended to a renal tumor implant model, which was used to show that the compound could also inhibit both primary tumor growth and the emergence of tumor metastasis to the lung. Using a non-invasive (color Doppler imaging) surrogate endpoint, a commensurate decrease in renal artery blood flow could also be observed after chronic treatment (Dreves *et al.*, 2000). Thus, PTK 787 appears to show significant preclinical efficacy, and to produce potent anti-tumor effects under well-tolerated dosing regimens. The preclinical toxicological profile and the human pharmacokinetics of this compound have not yet been disclosed.

Table 1 Selected small molecule tyrosine kinase inhibitors in clinical development for cancer

| Drug candidate       | Sponsor         | Target(s)   | Current/most advanced stage                     |
|----------------------|-----------------|---|---|
| STI 571 (CGP57148B)  | Novartis        | PDGFR, <i>abl</i> , <i>c-kit</i> , others?          | Phase III for CML                               |
| SU 101 (Leflunomide) | Pharmacia       | PDGFR, EGFR, pyrimidines                            | Phase II/III in multiple tumors (discontinued?) |
| Iressa (ZD1839)      | AstraZeneca     | EGFR  | Phase III for NSCLC, others                     |
| OSI-774 (CP-358,774) | Ocogene Science | EGFR  | Phase II for NSCLC, H/N                         |
| CI-1033 (PD183805)   | Pfizer          | EGFR and other erbB kinases                         | Phase I   |
| SU 5416              | Pharmacia       | VEGFR, PDGFR, others?                               | Phase II for NSCLC, colorectal ca               |
| SU 6668              | Pharmacia       | VEGFR, PDGFR, FGFR                                  | Phase I   |
| ZD4190, ZD6474       | AstraZeneca     | VEGFR (KDR plus <i>flt-1</i> )                      | Phase I   |
| PTK 787              | Novartis        | VEGFR (KDR plus <i>flt-1</i> ), PDGFR, <i>c-kit</i> | Phase I   |



## Development issues

It is clear that the development of newer agents like the tyrosine kinase inhibitors will require new concepts and clinical paradigms that are distinctly different from those used to develop the well-known cytotoxic agents commonly used in cancer chemotherapy. Some recent commentaries have done an outstanding job at framing these development issues (Sausville, 2000; Workman, 2000; Hudes, 1999; Eisenhauer, 1998).

Paramount among these is the need for non-conventional endpoints in clinical trial design, and for the identification, validation and implementation of surrogate endpoints which may help direct dose-modulation during therapy. From the examples provided above, it is clear that for several agents (Iressa, CP-358,774; SU 5416), the presumed efficacious dose in humans is very close to the maximally tolerated dose. None of these agents has yet been in a clinical trial designed to probe a broader aspect of efficacious dose range. Given the somewhat poor performance of preclinical efficacy models in predicting effective plasma concentrations in humans, organizations developing these new agents often resort to targeting some multiple of the plasma concentration required to generate efficacy in animal models, without first gaining an understanding as to whether clinical efficacy is dose-responsive, or that most patients are not being dosed at a level well-along on the plateau of the dose-response curve. Non-invasive approaches (Doppler and contrast agent imaging for VEGFR inhibitors) and invasive approaches (tumor and tissue sampling pre- and post-treatment for EGFR inhibitors) are being developed to aid in the assessment of minimally and maximally effective doses during the first days and weeks of clinical trial. The development of these surrogate endpoints is occurring on a parallel path with the agents themselves, probably too late to help define the dose-response, the minimally- and maximally-effective dose, or the most efficient development paradigm.

A second key issue is one of the biochemical selectivity of tyrosine kinase inhibitors, and the impact that it may have on both the efficacy and the safety of the clinical candidate. The current experience with both non-selective tyrosine kinase inhibitors (STI 571 and SU 5416) and selective compounds (Iressa® and CP-358,774) suggest that efficacy can be generated with either class of inhibitor, with modest, comparable safety margins. There could be an opportunity to assess the relative merits of EGFR-selective vs. pan erbB inhibitors when comparing the results of the trials with Iressa®, CP-358,774 and CI-1033, but the irreversibility of the latter candidate is likely to confound the comparisons of relative therapeutic index. A related issue is one of pre-screening patients for the over-expression of the target at which the tyrosine kinase is

directed. This may perhaps be a non-issue for VEGFR and PDGFR inhibitors, which target transiently, focally activated receptors on normal cells. However, it is clear that there may be different approaches in dealing with this issue during the development of EGFR inhibitors. AstraZeneca has apparently not incorporated prospective measurements of EGFR over-expression in tumors from patients with NSCLC as an inclusion criteria in their Phase III trials with Iressa®. As mentioned previously, one can surmise that the rationale for this strategy was based on both the high proportion of NSCLC tumors that over-express EGFR, and by preclinical data showing that over-expression is not predictive of a drug combination anti-tumor response.

Pfizer and Oncogene Science have executed at least one Phase II study with CP-358,774 in head and neck cancer patients where EGFR expression levels were evaluated as an entry criterion (Siu *et al.*, 2000). The basis of this strategy could be said to reside in the Genentech development experience with Herceptin®, in which all patients entered into clinical trial, and subsequently all patients receiving the approved commercial product, are first pre-screened to detect the level of over-expression of *erbB2* in their breast tumors. It is interesting to note that retrospective analyses attempting to relate the level of *erbB2* expression to clinical response in patients treated with Herceptin® (Dowsett *et al.*, 2000) have been inconsistent and unconvincing. It is somewhat troubling to see that so straight-forward an assay (immunohistochemistry) applied on a *post hoc* basis to understand an agent with so singular a mechanism of action (Herceptin®) has led to so little insight.

One can perhaps begin to gauge the obstacles that may lie ahead for the development of surrogate endpoints, which encompasses the application of novel technologies applied in a prospective way to drugs with complex mechanisms of action and pharmacological effects. This is the next frontier for the development of these new therapies for cancer. As such, the next 5–6 years are likely to be as challenging, and as exhilarating, as have been the past 5–6 years.

## Abbreviations

ATP, adenosine triphosphate; EGF/EGFR, epidermal growth factor/EGF receptor; PDGF/PDGFR, platelet-derived growth factor/PDGF receptor; CML, chronic myelogenous leukemia.

## Acknowledgments

I would like to acknowledge the outstanding contributions of each member of the Pfizer Global R&D division and the Pfizer Oncology team for their insights, support, commitment and good humor.

## References

- Allen LF, Cerna C, Gomez L, Yochmowitz M, Medina ML and Weitman S. (2000). *Proc. NCI-EORTC-AACR Symposium on New Drugs in Cancer Therapy*, 384.
- Ashman LK. (1999). *Intl. J. Biochem. Cell Biol.*, 31, 1037–1051.
- Barbacci EG, Cunningham A, Iwata K, Moyer JD and Miller PE. (1997). *Proc. Am. Assoc. Cancer Res.*, 38, 3143.

- Bartlett RR and Schleyerbach R. (1985). *Intl. J. Immunopharmacol.*, 7, 7–18.
- Baselga J, Herbst R, LoRusso P, Rischin D, Ranson M, Plummer R, Raymond E, Maddox A-M, Kaye SB, Kieback DG, Harris A and Ochs J. (2000). *Proc. Am. Soc. Clin. Oncol.*, 19, 686.

- Bold G, Altmann KH, Frei J, Lang M, Manley PW, Traxler P, Wietfeld B, Bruggen J, Buchdunger E, Cozens R, Ferrari S, Furet P, Hofmann F, Martiny-Baron G, Mestan J, Rosel J, Sills M, Stover D, Acemoglu F, Boss E, Emmenegger R, Lasser L, Masso E, Roth R, Schlachter C and Vetterli W. (2000). *J. Med. Chem.*, **43**, 2310–2323.
- Bonomi P, Perez-Soler R, Chachoua A, Huberman M, Karp D, Rigas J, Hammond L, Rowinsky E, Preston G, Ferrante KJ and Allen LF. (2000). *Proc. NCI-EORTC-AACR Symposium on New Drugs in Cancer Therapy*, 386.
- Bridges AJ. (1999). *Curr. Med. Chem.*, **6**, 825–843.
- Cherrington JM, Strawn LM and Shawver LK. (2000). *Adv. Cancer Res.*, **79**, 1–38.
- Ciardello F, Caputo R, Bianco R, Damiano V, Pomatico G, De Placido S, Bianco AR and Tortora G. (2000). *Clin. Cancer Res.*, **6**, 2053–2063.
- Cohen RB, Falcey JW, Paulter VJ, Fetzter KM and Waksal HW. (2000a). *Proc. Am. Soc. Clin. Oncol.*, **19**, 1862.
- Cohen S, Smolen J, Emery E, Cannon G, Weaver A and Schiff M. (2000b). *Arthr. Rheum.*, **43** (Suppl. 9), 1221.
- Connell RD. (2000). *Exp. Opin. Ther. Patents* **10**, 767–786.
- Cropp G, Rosen L, Mulay M, Langecker P and Hannah A. (1999). *Proc. Am. Soc. Clin. Oncol.*, **18**, 619.
- Dowsett M, Cooke T, Ellis I, Gullick WJ, Gusterson B, Mallon E and Walker R. (2000). *Eur. J. Cancer*, **36**, 170–176.
- Dreys J, Hofmann I, Hugenschmidt H, Wittig C, Madjar H, Muller M, Wood J, Martiny-Baron G, Unger C and Marmer D. (2000). *Cancer Res.*, **60**, 4819–4824.
- Druker BJ, Tamura S, Buchdunger E, Ohno S, Segal GM, Fanning S, Zimmermann J and Lydon NB. (1996). *Nat. Med.*, **2**, 561–566.
- Druker BJ and Lydon NB. (2000). *J. Clin. Invest.*, **105**, 3–7.
- Eckhardt SG, Rizzo J, Sweeney KR, Cropp G, Baker SD, Kraynak MA, Kuhn JG, Villalona-Calero M, Hammond L, Weiss G, Thurman A, Smith L, Drengler R, Eckhardt JR, Moczygemba J, Hannah AL, von Hoff DD and Rowinsky EK. (1999). *J. Clin. Oncol.*, **17**, 1095–1104.
- Eisenhauer EA. (1998). *Annals. Oncol.*, **9**, 1047–1052.
- Ferry D, Hammond L, Ranson M, Kris MG, Miller V, Murray P, Tullio A, Feyereislova A, Averbuch S and Rowinsky E. (2000). *Proc. Am. Soc. Clin. Oncol.*, **19**, 5E.
- Fong TAT, Shawver LK, Sun L, Tang C, App H, Powell TJ, Kim YH, Schreck R, Wang X, Risau W, Ullrich A, Hirth KP and McMahon G. (1999). *Cancer Res.*, **59**, 99–106.
- Fry DW. (2000). *Anti-Cancer Drug Design*, **15**, 3–16.
- Fry DW, Bridges AJ, Denny WA, Doherty A, Greis K, Hicks JL, Hook KE, Keller PR, Leopold WR, Loo J, Mcnamara DJ, Nelson JM, Sherwood V, Smail JB, Trump-Kallmeyer S and Dobrusin E. (1998). *Proc. Natl. Acad. Sci. USA*, **95**, 12022–12027.
- Fry DW, Kraker AJ, McMichael A, Ambrosio LA, Nelson JM and Leopold WR. (1994). *Science*, **265**, 1093–1095.
- Garber K. (2000). *J. Natl. Cancer Inst.*, **92**, 967–969.
- Ghosh S, Narla RK, Zheng Y, Liu X-P, Jun X, Mao C, Sudbeck EA and Uckun FM. (1999). *Anti-Cancer Drug Design*, **14**, 403–410.
- Gibbs JB. (2000). *J. Clin. Invest.*, **105**, 9–13.
- Goss G, Hirte H, Batist G, Stewart D, Miller W, Lorimer I, Abugaber A, Matthews S and Seymour L. (2000). *Proc. Am. Soc. Clin. Oncol.*, **19**, 880.
- Hamby JM and Showalter HDH. (1999). *Pharmacol. Ther.*, **82**, 169–193.
- Hamilton LC, Vojnovic I and Warner TD. (1999). *Br. J. Pharmacol.*, **127**, 1589–1596.
- Hammond LA, Denis LJ, Salman UA, Chintapalli K, Hidalgo M, Jeraback P, Patnaik A, Allen LF, Ferrante KJ, Carter WO, Kuhn, Drengler JR, Silberman S and Rowinsky EK. (2000). *Proc. NCI-EORTC-AACR Symposium on New Drugs in Cancer Therapy*, 385.
- Hanahan D and Weinberg RA. (2000). *Cell*, **100**, 57–70.
- Heinrich MC, Griffith DJ, Druker BJ, Wait CL, Ott KA and Zigler AJ. (2000). *Blood*, **96**, 925–932.
- Hudes G. (1999). *J. Clin. Oncol.*, **17**, 1093–1094.
- Hunter T. (2000). *Cell*, **100**, 113–127.
- Iwata K, Miller PE, Barbacci EG, Arnold LD, Doty J, DiOrio CI, Pustilnik LR, Reynolds M, Thelemann A, Sloan D and Moyer JD. (1997). *Proc. Am. Assoc. Cancer Res.*, **38**, 4248.
- Karp DD, Silberman SL, Csudae R, Wirth F, Gaynes L, Posner M, Bubley G, Koon H, Bergman M, Huang M and Schnipper LE. (1999). *Proc. Am. Soc. Clin. Oncol.*, **18**, 1499.
- Kelliher MA, McLaughlin J, Witte ON and Rosenberg N. (1990). *Proc. Natl. Acad. Sci. USA*, **87**, 6649–6653.
- Kelly HC, Ferry D, Hammond L, Kris M, Ranson M and Rowinsky E. (2000). *Proc. Am. Assoc. Cancer Res.*, **41**, 3896.
- Kuo EA, Hambleton PT, Kay DP, Evans PL, Matharu SS, Little E, McDowall N, Jones CB, Hedgecock CJ, YeaCM, Chan AW, Hairsine PW, Ager IR, Tully WR, Wwilliason RA and Westwood R. (1996). *J. Med. Chem.*, **39**, 4608–4621.
- Kurzrock R, Gutterman JU and Talpaz M. (1988). *N. Engl. J. Med.*, **319**, 990–998.
- Laird AD, Vajkoczy P, Shawver LK, Thurnher A, Liang C, Mohammadi M, Schlessinger J, Ullrich A, Hubbard SR, Blake RA, Fong TAT, Strawn LM, Sun L, Tang C, Hawtin R, Tang F, Shenoy N, Hirth KP, McMahon G and Cherrington JL. (2000). *Cancer Res.*, **60**, 4152–4160.
- Lawrence DS and Niu J. (1998). *Pharmacol. Ther.*, **77**, 81–114.
- le Coutre P, Mologni L, Cleris L, Marchesi E, Buchdunger E, Giardini R, Formelli F and Gambacorti-Passerini C. (1999). *J. Natl. Cancer Inst.*, **91**, 163–168.
- Levitzi A and Bohmer FD. (1998). *Anti-Cancer Drug Design*, **13**, 731–734.
- Levitzi A. (1999). *Pharmacol. Ther.*, **82**, 231–239.
- Luetke NC, Phillips HK, Qiu TH, Copeland NG, Earp HS, Jenkins NA and Lee DC. (1994). *Genes Dev.*, **8**, 399–413.
- Mattar T, Kochhar K, Bartlett R, Bremer EG and Finnegan A. (1993). *FEBS Lett.*, **334**, 161–164.
- Medina L, Gomez L, Cerna C, Kraker A, Yochmowitz M and S Weitman S. (2000). *Proc. Am. Assoc. Cancer Res.*, **41**, 3078.
- Mendel DB, Laird BD, Smolich BD, Blake RA, Liang C, Hannah AL, Shaheen RM, Ellis LM, Weitman S, Shawver LK and Cherrington JM. (2000). *Anti-Cancer Drug Design*, **15**, 29–41.
- Moyer JD, Barbacci EG, Iwata KK, Arnold L, Boman B, Cunningham A, DiOrio C, Doty J, Morin MJ, Moyer MP, Neveu M, Pollack VA, Pustilnik LR, Reynolds MM, Sloan D, Theleman A and Miller P. (1997). *Cancer Res.*, **57**, 4838–4848.
- Nelson JM and Fry DW. (2000). *Proc. Am. Assoc. Cancer Res.*, **41**, 1533.
- Oshervov N and Levitzi A. (1994). *Eur. J. Biochem.*, **225**, 1047–1053.
- Plate KH, Breier G, Weich HA, Mennel HD and Risau W. (1994). *Int. J. Cancer*, **59**, 520–529.
- Pollack VA, Savage DM, Baker DA, Tsaparikos KE, Sloan DE, Moyer JD, Barbacci EG, Pustilnik LR, Smolarek TA, Davis JA, Vaidya MP, Arnold LD, Doty JL, Iwata K and Morin MJ. (1999). *J. Pharmacol. Exp. Ther.*, **291**, 739–748.
- Randal J. (2000). *J. Natl. Cancer Inst.*, **92**, 520–522.
- Rosen L, Hannah A, Rosen P, Kabbinarav F, Mulay M, Gicanov N, DePaoli A, Cropp G and Mabry M. (2000). *Proc. Am. Soc. Clin. Oncol.*, **19**, 708.
- Rosen L, Mulay M, Mayers A, Kabbinarav F, Rosen P, Cropp G and Hannah A. (1999). *Proc. Am. Soc. Clin. Oncol.*, **18**, 618.

- Sausville EA. (2000). *Anti-Cancer Drug Design*, 15, 1–2.
- Sedlacek HH. (2000). *Drugs*, 59, 435–476.
- Shawver LK, Schwartz DP, Mann E, Chen H, Tsai J, Chu L, Taylorson L, Longhi M, Meredith S, Germain L, Jacobs JS, Tang C, Ullrich A, Berens ME, Hersh E, McMahon G, Hirth KP and Powell TJ. (1997). *Clin. Cancer Res.*, 3, 1167–1177.
- Sherwood V, Bridges AJ, Denny WA, Rewcastle GW, Smaill JB and Fry DW. (1999). *Proc. Am. Assoc. Cancer Res.*, 40, 723.
- Sirotnak FM, Zakowsky MF, Miller VA, Scher HI and Kris MG. (2000). *Proc. Am. Assoc. Cancer Res.*, 41, 3076.
- Siu LL, Hidalgo M, Nemunaitis J, Rizzo J, Moczygomba J, Eckhardt SG, Tolcher A, Smith L, Hammond L, Blackburn A, Tensfeldt T, Silberman S and von Hoff DD. (1999). *Proc. Am. Soc. Clin. Oncol.*, 18, 1498.
- Siu LL, Soulieres D, Senzer N, Agarwala S, Vokes E, Fisher D, Marsolais C, Ferrante KJ and Allen LF. (2000). *Proc. NCI-EORTC-AACR Symposium on New Drugs in Cancer Therapy*, 387.
- Smolen JS and Emery P. (2000). *Rheumatol.*, 39 (Suppl. 1), 48–56.
- Songyang Z and Cantley LC. (1998). *Methods Mol. Biol.*, 87, 87–98.
- Sungaran R, Chislom OT, Markovic B, Khachigian LM, Tanaka Y and Chong BH. (2000). *Blood*, 95, 3094–3101.
- Talpaz M, Sawyers CL, Kantarjian H, Resta D, Fernandes Reese S, Ford J and Druker BJ. (2000). *Proc. Am. Soc. Clin. Oncol.*, 19, 6.
- Thompson WD, Li WW and Maragoudakis M. (1999). *J. Pathol.*, 187, 503–510.
- Tzahar E, Waterman H, Chen XM, Levkowitz G, Karunagaran D, Lavi S, Ratzkin BJ and Yarden Y. (1996). *Mol. Cell. Biol.*, 16, 5276–5287.
- Vincent PW, Patmore SJ, Atkinson BE, Bridges AJ, Kirkish LS, Dudeck RC, Leopold WR, Zhou H and Elliott WL. (1999). *Proc. Am. Assoc. Cancer Res.*, 40, 117.
- Wakeling AE, Barker AJ, Daview DH, Brown DS, Green LR, Cartledge SA and Woodburn JR. (1996). *Breast Cancer Res. Treat.*, 38, 67–73.
- Wang W-L, Healy ME, Sattler M, Verma S., Lin J, Maulik G, Stiles CD, Griffin JD, Johnson BE and Salgia R. (2000). *Oncogene*, 19, 3521–3528.
- Ward WHJ, Cook PN, Slater AM, Daview H, Holdgate GA and Green LR. (1994). *Biochem. Pharmacol.*, 48, 659–666.
- Wedge SR, Waterton JC, Tessier JJ, Checkley D, Dukes M, Kendrew J and Curry B. (1999). *Proc. Am. Assoc. Cancer Res.*, 40, 2741.
- Wedge SR, Ogilvie DJ, Dukes M, Kendrew J, Curwen, JO, Hennequin F, Thomas AP, Stokes ESE, Curry B, Richmond GHP and Wadsworth PF. (2000a). *Cancer Res.*, 60, 970–975.
- Wedge SR, Ogilvie DJ, Dukes M, Kendrew J, Hennequin F, Stokes ESE and Curry B. (2000b). *Proc. Am. Assoc. Cancer Res.*, 41, 3610.
- Wood JM, Bold G, Buchdunger E, Cozens R, Ferrari S, Frei J, Hofmann F, Mestan J, Mett H, O'Reilly T, Persohn E, Rosel J, Schnell C, Stover D, Theuer A, Towbin H, Wenger F, Woods-Cook K, Menrad A, Siemeister G, Schirner M, Thierauch K-H, Schneider MR, Dreys J, Martiny-Baron G, Totzke F and Marme D. (2000). *Cancer Res.*, 60, 2178–2189.
- Woodburn JR, Barker AJ, Gibson KH, Ashton SE, Wakeling AE, Curry BJ, Scarlett L and Henthord LR. (1997). *Proc. Am. Assoc. Cancer Res.*, 38, 4251.
- Workman P. (2000). *Curr. Opin. Oncol., Endocrin. Metab. Invest. Drugs*, 2, 21–25.
- Xu X, Williams JW, Bremer EG, Finnegan A and Chong AS-F. (1995). *J. Biol. Chem.*, 270, 12398–12403.